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NOMENCLATURE OF THE PROTEINS OF BOVINE MILK—FIRST REVISION

Report of the Committee on Milk Protein Nomenclature, Classification, and Methodology of the Manufacturing Section of A.D.S.A. for 1958-59

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SUMMARY

The α-casein component of the casein system of milk, which appears as a single, leading electrophoretic peak at pH 8.6, is heterogeneous and should be referred to as the α-casein fraction. The components comprising this fraction vary in distribution, dependent upon the experimental conditions. *Alpha*-casein has been separated into calcium-sensitive and calcium-insensitive fractions which have been designated by various symbols. These fractions usually are in the form of an α (Ca sensitive)—α (Ca insensitive) complex in equilibrium with its components. In view of the complexity of the α-casein fraction, this Committee feels that no recommendations on nomenclature should be made at this time. Cherbuliez's δ-casein, Hammersten's proteose, and the 2% and 12% TCA-soluble peptides of Alais and Nitschmann, materials apparently derived from α-casein by various procedures, are discussed in this report.

β-lactoglobulin, obtained from mixed milk, is composed of at least two forms of β-lactoglobulin which are genetically determined and referred to as β-lactoglobulins *A* and *B*, discernible by paper electrophoresis in veronal buffer at pH 8.6, where Type *A* constitutes the leading component. Further, Type *A* associates in acetate buffer between pH 3.7 and 5.2 and is essentially monomeric at pH values alkaline to its isoelectric point, whereas Type *B* exists in its monomeric form under similar conditions. These characteristics explain in part the electrophoretic and ultracentrifugal heterogeneity of normal (mixed) β-lactoglobulins *A* + *B* and β-lactoglobulin *A*.

A previous Committee report, Jenness *et al.* (22), published in 1956, contributed materially to the clarification of a rapidly expanding system of nomenclature for the proteins of bovine skimmilk. The electrophoretically discernible protein components were categorized in terms of their classical or more traditional nomenclature and in relationship to the contemporary nomenclature. Although electrophoretic resolution of the protein components in free-boundary electrophoresis was selected as a convenient criterion for classification, the possibility was recognized that many of these so-called individual proteins were actually complexes or heterogeneous mixtures of proteins possessing similar electrophoretic characteristics at pH 8.6.

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Recent investigations have demonstrated the heterogeneity of the α-casein and β-lactoglobuliln fractions. New, and at times confusing, terminology has been introduced by investigators to identify the proteins they have reported.

The present Committee, in an attempt to cope with the problem of expanding terminology, recognized that: Within reasonable limits, the prerogative of the investigator to assign an appropriate nomenclature to the proteins he has isolated and/or characterized should be preserved, provided that he shows conclusive evidence that his protein differs in fact, not purity, from any protein previously isolated and characterized; and the Committee's principal function was the resolution of newly introduced terminology in terms of contemporary nomenclature rather than recommending prematurely a rigid nomenclature system. Conceivably, newly reported protein fractions of similar characteristics could be classified tentatively pending: (a) the presentation of additional confirmatory evidence in support of the homogeneity of the protein, and (b) the development of a sound nomenclature system for milk proteins. An awareness of the progress being made in this field would indicate that a stabilized nomenclature system must await a more complete elucidation of the milk protein system.

Based upon the foregoing considerations, this Committee has considered the terminology introduced by investigators to designate the components of the α-casein fraction and β-lactoglobulin and has presented a compilation of this terminology in terms of contemporary usage. Also, certain additions and revisions have been made to the table of characteristics published in the 1956 report. In so doing, the Committee realized that further development will necessitate additional revisions, until a more complete elucidation of the milk protein system makes possible the establishment of a nomenclature and classification system which will facilitate research in the field of milk proteins.

Casein. The 1956 report recognized the individuality of the components α-, β-, and γ-casein in the protein fraction precipitated by acidifying raw skimmilk to pH 4.6. A fourth component, δ-casein, isolated by Cherbuliez and his coworkers (7, 8, 9) and characterized by its solubility in 10% TCA, was considered as a possible entity. The similarity between δ-casein and Hammersten's proteose, found as a product of the reaction of rennet on casein, was postulated (10). Alais (1) showed similarities between Hammersten's proteose, Cherbuliez's δ-casein, and a 2% TCA-soluble fraction obtained by the action of crystalline rennin on casein. The bulk of the 2% TCA-soluble fraction stems from the α-casein fraction (2) and more specifically from the calcium-insensitive fraction (κ-casein) (57, 59) by the primary action of rennin. The fact that Cherbuliez and Baudet (8) did not isolate δ-casein from paracasein suggests further that δ-casein and the rennin-liberated proteins may have similar origins. Further investigations are required to establish the classification of δ-casein.

Within recent years several papers have been published elucidating the α-casein fraction. Waugh and von Hippel (61) were the first to show that α-casein could be separated into calcium-sensitive [α_s-casein (59)] and calcium-insensitive [crude-κ-casein] fractions, based upon its dissociation and differential solubility in the presence of calcium ions. The fractions of casein not classified as as α_s-, crude-κ-, or β-casein were designated *m* fraction (60). Long *et al.* (30), working along a similar approach, were able to isolate a slow sedimenting fraction from Waugh's second cycle, crude-κ-casein, characterized by its high phosphorus content (1.1%), which they called λ-casein. They suggested that the calcium-insensitive fraction of Waugh possessed properties similar to the ζ-casein fraction isolated by Linderstrøm-Lang (29). The calcium-sensitive fraction of the α-casein fraction was called α_R-(30) and, in a previous study, α_P-casein (44). Wake (57) has referred to the calcium-sensitive fraction as α-casein.

McMeekin *et al.* (33) reported the isolation of a fraction from α-casein possessing minimum solubility at pH 5.8-6.0, but soluble at pH 4.7 as well as pH 4.0 (32), which they called α_2-casein. This fraction was obtained from acid casein in yields approximating 1%, was low in phosphorus content (0.1%), not precipitated by calcium ions nor clotted by rennet, but was split by rennet at pH 7.3 into soluble and insoluble fractions. The remaining and larger fraction was called α_1-casein. Electrophoretic mobilities for the α_1- and α_2-casein fractions in veronal buffer at pH 8.4, $\Gamma/2 = 0.1$ were -6.7 and -5.0, respectively. McMeekin (31, 32) has since referred to the α_2-casein fraction as α_z-casein. Characteristics of α_z-casein suggest its similarity to Linderstrøm-Lang's Z-casein and Waugh's κ-casein. Further, McMeekin (33, 34) referred to the calcium-sensitive fraction of α-casein as α_1-casein. More recently, Hipp *et al.* (19) have reported the isolation of α_3-casein, characterized by a single ultracentrifugal component at pH 7.1 ($S_{20} = 23$ in phosphate buffer) and by its resemblance to κ-casein in physical properties.

Cherbuliez and Baudet (7) fractionated α-casein into two fractions by warming it to 40° C. in 5% $(NH_4)_2SO_4$ solution. The soluble (α_I) and insoluble (α_{II}) fractions were similar in composition and can not be considered as different protein entities.

Nitschmann and Lehmann (37) showed that rennin, when added to sodium α-caseinate, induced an electrophoretically discernible split in the alpha component; the two peaks were called α_1 and α_2 in descending order of mobility. Payens (40), studying the action of rennin on Waugh's first- and second-cycle casein fractions, concluded that Nitschmann's α_2--casein was quite similar, if not identical, to Waugh's *kappa*-rich fraction. The split in the electrophoretic peak of the α-casein fraction, which has been noted by many investigators, indicates the presence of more than one component and presents data sometimes difficult to interpret, since any one or a combination of factors, i.e., individual differences among cows, variations in ionic strength, freezing or dehydration of the casein preparation, and prolonged storage of casein solutions, could contribute to the occurrence of such an observation (12, 58).

Obviously, the α-casein fraction consists of a highly integrated system composed of calcium-sensitive and calcium-insensitive protein components. Actually, a precise interpretation of the data reported in support of the various casein fractions is most difficult because of the tendency of casein components to form aggregates under all except very drastic conditions (20, 36). For example, it is difficult to know whether a fraction with given properties is a pure component, a mixture of components, or a fraction reflecting the specific conditions used in its isolation. Aggregation makes moleculer weight determinations especially troublesome and uncertain.

In view of these advancements in the knowledge of the casein system, the classical term calcium *para*-caseinate should be redefined to indicate the specificity of the action of rennin (14, 57, 59, 50). Waugh et al. (59, 60) have proposed a new terminology for the action of rennin on the α-casein fraction: *para*-κ-casein for the primary reaction product of rennin on κ-casein and α_s-*para*-κ-casein for the clots which are formed. The role of other α-casein components and of β- and/or γ-casein in this transformation has not been elucidated.

No recommendation relative to the nomenclature of the α-casein complex is suggested at the present time. As reviewed above and in Table 2, the α-casein complex is currently the subject of a great deal of research. It appears desirable to withhold a recommended nomenclature for the α-casein complex until the components and physical/chemical equilibria involved are more precisely and completely understood.

β-lactoglobulin. Pedersen's (41) electrophoretic and ultracentrifugal studies with Palmer's β-lactoglobulin led him to believe that the protein was homogeneous. Li (28), employing more sensitive electrophoretic techniques, observed that β-lactoglobulin showed a single electrophoretic peak in acetate buffer at pH 5.3 and 5.6, but showed three components when observed in buffer at pH 4.8 and 6.5. In both cases, the fastest-moving boundary constituted the major portion of the pattern and had an isoelectric point of 5.1. Polis *et al.* (43) found that β-lactoglobulin isolated by alcohol fractionation and differential solubility at pH 4.8 and 5.3 from a pooled milk supply showed a single electrophoretic peak in buffers alkaline to the isoelectric point and two maxima in buffers on the acid side. They isolated the slow-moving fraction (pH 4.8) in small yields, which they designated β_1-lactoglobulin. The fast-moving component, which was not purified, was termed β_2-lactoglobulin. Aschaffenburg and Drewry (4) prepared β-lactoglobulin-rich fractions from the milk of individual cows, which they studied by paper electrophoresis in veronal buffer at pH 8.6, $\Gamma/2 = 0.05$. They observed that individual animals gave milk containing one or the other or a mixture of both of two electrophoretically discernible β-lactoglobulins. They designated the faster of the two components β_1-lactoglobulin and the slower as β_2-lactoglobulin.

Ogston and Tombs (38), working with β-lactoglobulin crystallized from the milk of individual cows, found that β_1-lactoglobulin, the fastest-moving component on filter paper electrophoresis at pH 8.6, was also the fastest component observed during free-boundary electrophoresis in acetate buffer at pH 4.6. This observation caused them to regard the protein isolated by Polis as a subfraction, not related to the β_1-lactoglobulin observed by Aschaffenburg and Drewry (5). In addition, they observed that both β_1- and β_2-lactoglobulin were electrophoretically heterogeneous at pH 4.6. Klostergaard and Pasternak (23) observed that the mobility of Polis' β_1-lactoglobulin was slower than Aschaffenburg's β_1-lacto-

TABLE 1

Protein fractions of bovine skimmilk and some of their properties

Protein fraction: Classical nomenclature[a]	Protein fraction: Contemporary nomenclature	Occurrence in electrophoretic pattern[b] (Peak No.)	Reference to preparation	Approximate per cent of skimmilk protein[c]	Sedimentation constant[d] (S_{20})	Molecular weight[e]	PI[f]	Electrophoretic mobility at pH 8.6[g]	Other characteristics
Casein (precipitated from skimmilk by acid at pH 4.6)			18, 58	76-86	1.18 (20)[i]	15,000 (20) 33,600 (6)			
	α-casein	In casein pattern (17) 1	18, 58	45-63	3.99 (50)	27,000 (36)	4.1 (22)	−6.7 (22)	Contains 1% phosphorus. Consists of a mixture of interesting proteins (see Table 2). Formed in the udder[n]
	β-casein	2	18, 58	19-28	1.57 (50)	24,100 (50)	4.5 (22)	−3.1 (22)	0.6% Phosphorus. Formed in udder
	γ-casein	3	18	3-7	1.55 (35)	30,600 (35)	5.8-6.0 (22)	−2.0 (22)	0.1% Phosphorus. Preformed from blood
Noncasein proteins		In acid whey pattern (27)		14-24					
Lactalbumin (Soluble in saturated $MgSO_4$ soln.)	β-Lactoglobulin A	6	4		2.8 (53)[i]	35,000 (38)		−5.3 (54)	Associates in pH range 3.7 to 5.3. Formed in udder
	β-Lactoglobulin B (Mixed A and B)	6 39	4	7-12[h]	5.3 (53)[j] 2.7 (53)	35,000 (38)		−5.2 (54)	Exists principally in monomeric form. Formed in udder.
	α-Lactalbumin	4	15, 16	2-5	1.75 (15)	16,500 (15)	5.1 (24)[l]	−4.2 (15)[m]	7% tryptophane. Formed in udder
	Blood serum albumin	7	42	0.7-1.3	4.0 (11)	69,000 (42)	4.7 (42)	−6.7 (42)	Apparently identical to bovine serum albumin. Preformed from blood

Lactoglobulin (Insoluble in saturated $MgSO_4$ soln.)	Euglobulin	1	48	0.8-1.7	8.77 (35)[k]	252,000 (35)[k] 180,000 (48)[k]	6.0 (35)	−1.8 (35)	Fractions containing antibodies. Contain hexose and hexosamine. Electrophoretically and ultracentrifugally heterogeneous. Preformed from blood.
	Pseudoglobulin	2	48	0.6-1.4	8.07 (35)[k]	289,000 (35)[k] 180,000 (48)[k]	5.6 (35)	−2.0 (35)	
Proteose-Peptone Fraction (Not precipitated at pH 4.6 from skimmilk previously heated to 95–100° C., 30 min.)		3 5 8	3, 22, 27	2-6	0.96 (3)[k] 2.75 (3) 1.0 (22)	4,900 (3)[k] 24,000 (3)		−3.0 (27) −4.6 (27)[k] −7.9 (27)	Glycoprotein (51). Electrophoretically and ultracentrifugally heterogeneous. Poorly defined except for serum component 5 (21).

[a] Rowland fractions (22, 46, 47).

[b] Free-boundary electrophoresis in veronal buffer at pH 8.6, $\Gamma/2 = 0.1$. Casein components designated in descending order of mobility. Serum protein components designated in ascending order of mobility.

[c] Values compiled and/or calculated from Rowland nitrogen distribution data, relative areas of electrophoretic patterns, and protein yield studies (18, 22, 27, 45, 46, 47).

[d] s_{20} = sedimentation constant = $(dx/dt)\ (1/w^2x)$, in Svedberg units ($S = 1 \times 10^{-13}$) corrected to 20° C. See original literature for experimental conditions. Sedimentation characteristics are dependent upon ionic strength of solvent, temperature, pH, and concentration of solute. The sedimentation and molecular weight values reported are not necessarily the best values obtainable, nor do they constitute endorsement by the Committee.

[e] Refer to original literature for method and conditions of determination.

[f] Isoelectric point, i.e., pH of no electrophoretic movement.

[g] Electrophoretic mobility (u) = $\times 10^{-5}$, cm.^{2}, volts^{-1}, sec.$^{-1}$ obtained by the Tiselius moving boundary method at 2° C. in veronal buffer at pH 8.6, $\Gamma/2 = 0.1$. Measured from descending pattern.

[h] Distribution of β-lactoglobulin A and B are genetically determined (4, 5).

[i] Denotes the characteristic of the monomeric specie.

[j] Denotes the characteristic of the associated specie.

[k] Denotes the characteristics of the major component.

[l] Value replaces previously reported value of 4.1 to 4.8.

[m] Mobility reported at −3.6 in milk serum protein mixture (26).

[n] Source of information pertaining to the origin of the milk proteins (25).

TABLE 2

Reported fractions or components of α-casein and some of their properties[a]

Contemporary nomenclature	Occurrence in electrophoretic pattern (Peak No.)	Reference to preparation	Approximate per cent of skimmilk protein	Sedimentation constant (S_{20})	Molecular weight	PI	Electrophoretic mobility at pH 8.6	Other characteristics
α-casein[b]								
Ca-sensitive component(s)	(1)							
α_s-casein		61[c]	37-54 (61)	1.59 (61)[g]	23,300 (59)			1.10% phosphorus
α_1-casein		33[c]		3.0 (34)		4.3-4.7 (34)	−6.7 (33)	0.85% phosphorus
α_R-casein		30[c]		4.55 (30)				1.16% phosphorus
Ca-insensitive component(s)	(1)		(49, 57, 61)					
κ-casein		13, 61[d,e]	11-13	1.4 (61)[g] 13.5 (61)[h]	16,300 (59)			0.19 to 0.33% phosphorus Stabilizes α_s-casein to Ca ions
α_2-or α_s-casein		33[e]					−5.0 (33)	0.1 to 0.15% phosphorus. Isolated in small yields. Soluble at pH 4.7. Resembles κ-casein.
α_3-casein		19[e]		23.0 (19)				Stabilizes α_1-casein to Ca ion.
λ-casein		30[f]	1.2 (30)	1.1 (30)				1.18% phosphorus, not stabilizing.
m casein		59, 60[f]						

[a] Units and experimental conditions similar to those described for Table 1.
[b] Method of isolation from whole casein can determine content of calcium-sensitive components (62).
[c] Similar characteristics suggest that proteins are similar.
[d] Gross calcium-insensitive fraction.
[e] Similar characteristics suggest that proteins are similar.
[f] Similar characteristics suggest that proteins are similar.
[g] Denotes characteristics of monomeric form.
[h] Denotes characteristics of associated form.

globulin on paper electrophoresis at pH 8.6, indicating that Polis' β_1-lactoglobulin corresponded to Aschaffenburg's β_2. This observation has since been confirmed in free-boundary electrophoresis by Timasheff and Townend (54).

Aschaffenburg and Drewry (5) discovered that the secretion of β_1-lactoglobulin and β_2-lactoglobulin was under genetic control. This induced them to abandon the old nomenclature in favor of the β-lactoglobulin-*A* and *B* nomenclature which is more acceptable from a genetic standpoint. β-lactoglobulin *A* and *B* are discernible in descending order of electrophoretic mobility in veronal buffer at pH 8.6.

Recent work of Timasheff and Townend (52, 53, 54, 55) contributed significantly to the characterization of β-lactoglobulins *A* and *B*. The electrophoretic mobility at pH 4.65 of β-lactoglobulin *B* was slower than the polymer Type A, but slightly faster than the monomer. They concluded, from supporting ultracentrifugal data, that the observed association of β-lactoglobulin in the pH range of 3.7 to 5.2 was due to β-lactoglobulin *A*. Type *B* sedimented as a single molecular specie at concentrations up to 7%. The conclusions supported the work of Ogston and Tombs (38, 56), who suggested that the association of β-lactoglobulin was due to the aggregation or association of Type *A*.

The following recommendations relative to the nomenclature of the β-lactoglobulins are suggested: β-lactoglobulin exists in two forms which are genetically defined and discernible by paper electrophoresis at pH 8.6. These were designated by Aschaffenburg and Drewry (5) as β-lactoglobulin *A* (faster-moving component) and *B* which appears to be the accepted designation for reasons mentioned earlier. β-lactoglobulin *A* associates in the range of pH 3.7 to 5.2, while β-lactoglobulin *B* exists principally in a monomeric state. This particular characteristic of the β-lactoglobulins manifests itself in the heterogeneity observed in the electrophoretic and ultracentrifugal patterns of β-lactoglobulin *A* and *B* (mixed) and β-lactoglobulin *A*, when studied within this pH range. The β-lactoglobulins are essentially monomeric in alkaline media.

The foregoing discussions serve as background material upon which the deliberations of this Committee were based. The present status of the available knowledge relating to the characteristics of the protein fractions of milk presently known as α-casein and β-lactoglobulin has induced us to suggest revisions to the report of the 1956 Committee (see Tables 1 and 2). Obviously, the voids in data within these tables demonstrate the paucity of our information relating to physical and chemical properties of skimmilk proteins. Also, the Committee wishes to point out a certain lack of evidence in support of the homogeneity of fractions or components listed under α-casein, and that inclusion in the table does not constitute unmitigated acceptance of the component as a protein entity, but, rather, a compilation of the current information relating to the characteristics and nomenclature of the α-casein fraction. In some instances, apparently, similar components have been designated differently by the various authors. The Committee hopes that by compiling these data, it willl, to some degree, stimulate continued studies toward the elucidation of the milk protein system

Free-boundary electrophoresis continues to be recognized as a primary standard for classification in the nomenclature scheme, but it is apparent that other physical and chemical criteria are required to elucidate the complexity of electrophoretically homogeneous components.

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